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OBSERVATIONS UPON *TRICHOMONAS INTESTINALIS IN VITRO*

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In a recent communication to this Journal I reported a successful cultivation of a strain of *Trichomonas intestinalis*. It is the purpose of the present communication to record some further observations upon this intestinal flagellate.

Fecal suspensions for subculturing the strain have been continuously secured from the same individual, but I have found that certain specimens of feces are unsuitable for the purpose of making suspensions, since in these the organisms will not grow. These unsatisfactory suspensions are characterized by an acid reaction to litmus and an intense bile staining of the saline above the fecal sediment. On the other hand, the suspensions in which good growth has been secured have been neutral to litmus and the supernatant saline has been colorless or only tinged slightly yellow.

I now prepare the fecal suspension as follows:

A soft portion of the fecal mass is selected and thoroughly triturated in about ten volumes of saline and strained through cheese cloth to remove the coarser particles of fecal debris. The suspension is then centrifuged until the supernatant fluid is clear. If not bile stained and if its reaction is neutral, about one cubic centimeter of the sediment is added to each of a number of test tubes containing about ten cubic centimeters of physiological saline. Without sterilization these are employed for subculturing, one or more being reserved as controls to indicate that flagellates are not present in the feces employed in making the suspension. If the supernatant wash fluid were bile stained it might be possible to secure a satisfactory suspension by one or more washings to remove the excess of bile, but so far I have not had to do this. The suspension thus consists of living fecal bacteria and small particles of undigested or partially digested food. The bacteria prey on the latter and the trichomonads prey on the former. In old cultures there is a very noticeable reduction in both the bacterial and food debris.

Since its cultivation *in vitro* our strain of *Trichomonas* has been carried through subcultures as follows:

No.	Source	Age of Parent Subculture at Time, Days	Greatest Age at which Motile Flagellates Found, Days	Greatest Age at which Subcultures Gave Growth, Days
1	Original	..	59	60
2	1	13	46	47
3	2	18	28	65
4	Failure*	..	..	..
5	3	29	27	..
6	5	24	4	..
6	3	65	9	..
7	6	9	..	..

*Probably due to excess of bile in feces.

From the foregoing it may be seen that these cultures retain their vitality, as measured by the presence of active flagellates, for a period which compares favorably with those of some of the more rugged pathogenic bacteria when growing *in vitro*. Not much growth is evident within the forty-eight hours following a transfer, but it apparently reaches a maximum when the culture is from four to five days old. A loopful of the sediment, placed on a slide and flattened out with a cover glass, may with the low power, reveal nearly a hundred flagellates per field. Following this, their numbers undergo a steady diminution, until not over two or three will be observed in an entire preparation made from a loopful of sediment. I have reason to believe that this diminution in the numbers of flagellate forms is due to encystment.

My observations have convinced me that this organism is *Trichomonas intestinalis,* though as noted before, I have failed to observe an axostyle in the fixed preparations I have made. Nevertheless, an axostyle is distinctly visible in the living organism, projecting posteriorly as a rigid spinous process, as shown in the outline drawings in the plate herewith. The axostyle apparently serves as an organ for temporary attachment. I have observed individuals attached by its assistance to the cover glass or food debris twist and rotate upon their axostyle very much in the manner of a vorticella. When fully active the three anterior flagella move at such a rapid rate they cannot be seen. However, in sluggish individuals they are distinctly visible, and it can be seen that their movement partakes of a rotary character, as indicated in Figure 4. The anterior portion of the undulating membrane is rarely visible during life, but the posterior portion, apparently having a longer membrane, can readily be seen. In very sluggish individuals its movements are suggestive of the movements of pseudopodia, especially where the flagellate is in the pre-encysting stage. From observations of living organisms, it seems probable that the idea these organisms may project pseudopodia, as suggested by Wenyon (1915) may have arisen from observation of the movements of the posterior portion of the undulating membrane in sluggish individuals. In the pre-encysting state, when the organism, deprived of all appendages, seems to possess a distinct ectoplasmic-endoplasmic differentiation and thus somewhat resembles a small amoeba, the sluggish movements of the undulating membrane are still more suggestive of pseudopodia. However, the ectoplasmic bulgings, representing the altered undulating membrane, first appear toward the anterior extremity and slowly progress posteriorly. These are indicated at the arrows in Figures 7, 8 and 9.

In stained preparations the organism is seen to possess three anterior flagella, and a single posterior flagellum, having an origin at the same place as the others, but projecting posteriorly and forming the free edge of the undulating membrane. It may extend beyond the posterior extremity of the cell body as a free flagellum. A distinct chromatic line at the base of the undulating membrane is visible. All four flagella arise from an anterior blepharoplast or kinetonucleus. The cytostome has not been observed in any of the individuals examined, neither have chromatic blocks been observed about the nucleus. The interior of the cell body invariably contains food vacuoles of varying size, in many of which can be observed bacteria in various stages of digestion. As previously noted, I have not observed the axostyle in fixed specimens, though it is clearly visible in the living animal.

Multiplication appears to be by longitudinal division, an example of which is indicated in Figure 5, in which there can be observed two nuclei, two sets of flagella and two chromatic lines, but cleavage of the cell body has not yet occurred.

In an endeavor to see if the culture strain could re-establish itself in an animal, two white rats were selected, and by fecal examination determined to be free from trichomonads. One rat (A) was given one feeding of milk to which had been added a portion of Culture 2, at that time forty-two days old. Not over one motile flagellate was present per slide preparation, while the culture chiefly contained the spherical bodies later recognized as cysts. The other rat (B) was kept in another cage as a control, receiving similar feed and treatment, but was not fed infected food. Six days later a single motile trichomonad was observed in preparations made from a fecal pellet of Rat A, while similar preparations from Rat B were negative. The entire remaining fecal pellets from both rats were transferred to tubes of saline and separately suspended therein. Three days later these cultures were examined, and from four to five trichomonads were observed per field in the culture from the feces of Rat A, while none were present in the culture from Rat B. Fourteen days after the infected feeding, both rats were killed. The results of the examination of Rat A (infected) were as follows: No flagellates were observed in the feces, but spherical bodies regarded as cysts were present; the cecum was full of soft pultaceous feces, which was literally swarming with *myriads* of trichomonads; no trichomonads were observed in the small intestine or stomach. Examination of Rat B (control) was entirely negative for trichomonads. The cecal contents of both rats were transferred to saline. When examined three days later, numerous trichomonads were present in the culture from Rat A, but in numbers much less than in the fresh cecal contents. The culture from Rat B was negative. Motile flagellates were still present in the culture from A when examined a month later.

The mucosa lining the cecum of Rat A appeared perfectly normal and throughout the course of the experiment the rat appeared to enjoy unimpaired health.

In studying the growth in cultures I had at first difficulty in recognizing the trichomonas cysts. Various observers have described objects they regarded as trichomonas cysts, a number of which are figured by Lynch, but the chief claim in favor of each appears to be the fact they have been observed in material in which trichomonads were present, and hence were assumed by the observers to be cysts of the observed protozoan. These conflicting views were found confusing. I finally found that by staining the living trichomonads with dilute neutral red solutions and allowing the preparations containing them to undergo gradual desiccation, cyst formation invariably took place and all stages the process could be observed. Figures 7 to 14 represent the process of cyst formation from the flagellate stage in one individual, while Figures 15, 16 and 17 represent typical cysts. The cysts are perfectly spherical and from 5 to 6 micra in diameter. In some a peripherially located nucleus can be discerned. In the drawings the relative thickness of the double contoured wall is somewhat exaggerated. In cultures, without the aid of neutral red solutions to stain the food vacuoles, the cysts can only be made out with difficulty, since their walls are not highly refractile. I am not satisfied I have as yet observed them in fixed films.

It remains to be ascertained whether infection by feeding results from the flagellates or from the cysts. By analogy the latter would seem probable and my single feeding experiment, taking into consideration the paucity of flagellates present, would tend to support this view.

Reference Cited

Wenyon, C. M. 1915.—Observations on the Common Intestinal Protozoa of Man. London Lancet, 2: 1172, Nov. 27.

EXPLANATION OF PLATE

All figures are free hand drawings

Figs. 1, 2, 3, 4. Unstained living trichomonads. 1, 2, and 3 show various changes of position of a trichomonad which has temporarily attached itself to the coverslip by the tip of its axostyle. Movements consist of a rotation on its long axis as well as lateral flexion. Flagellar movement is too active for visibility. 4, outline of a trichomonad whose movements were sluggish. The three anterior flagella rotate as indicated by the arrows. At (*a*) the movements of the undulating membrane are most distinct. When movement of the membrane temporarily ceases, the change in the organism's shape is indicated by the dotted line.

Figs. 5, 6. From smears fixed in sublimate alcohol and stained with Heidenhain's iron hematoxylin. 5, beginning longitudinal division. Cleavage of the cell body has as yet not taken place. 6, a typical trichomonad.

Figs. 7 to 17. Cyst formation. Living trichomonads from culture stained with dilute neutral red. The food vacuoles stain a dark cherry red and the nucleus appears as a pale pink disk. Figs. 7-14, progressive changes in cyst formation. Changes observed in a single individual. 7. Pre-encysting form. Sluggish, no flagella observable, movements of undulating membrane, indicated by arrow, very sluggish and resemble pseudopodia. 12:20 p. m. 8. Some contraction of the body. Axostyle retracted. Undulating membrane still moving. 12:50 p. m. 9. Distinct differentiation into ecto- and endo-plasm. Undulating membrane moving slightly. 1:15 p. m. 10. Some extension of ectoplasm, approaching original shape. 1:40 p. m. 11. Ectoplasm again contracting. 1:45 p. m. 12. Nucleus no longer visible. Outline nearly spherical. 1:50 p. m. 13. Peripheral ectoplasm becomes denser, outline distinctly double contoured. 1:52 p. m. 14. Perfectly spherical, double contoured wall. Clear zone of ectoplasm has disappeared. Cyst complete. 1:57 p. m.

Figs. 15, 16, 17. Typical cysts. Nucleus in peripheral situation is visible in 15. Food vacuoles visible in all. Diameter from 5 to 6 micra.

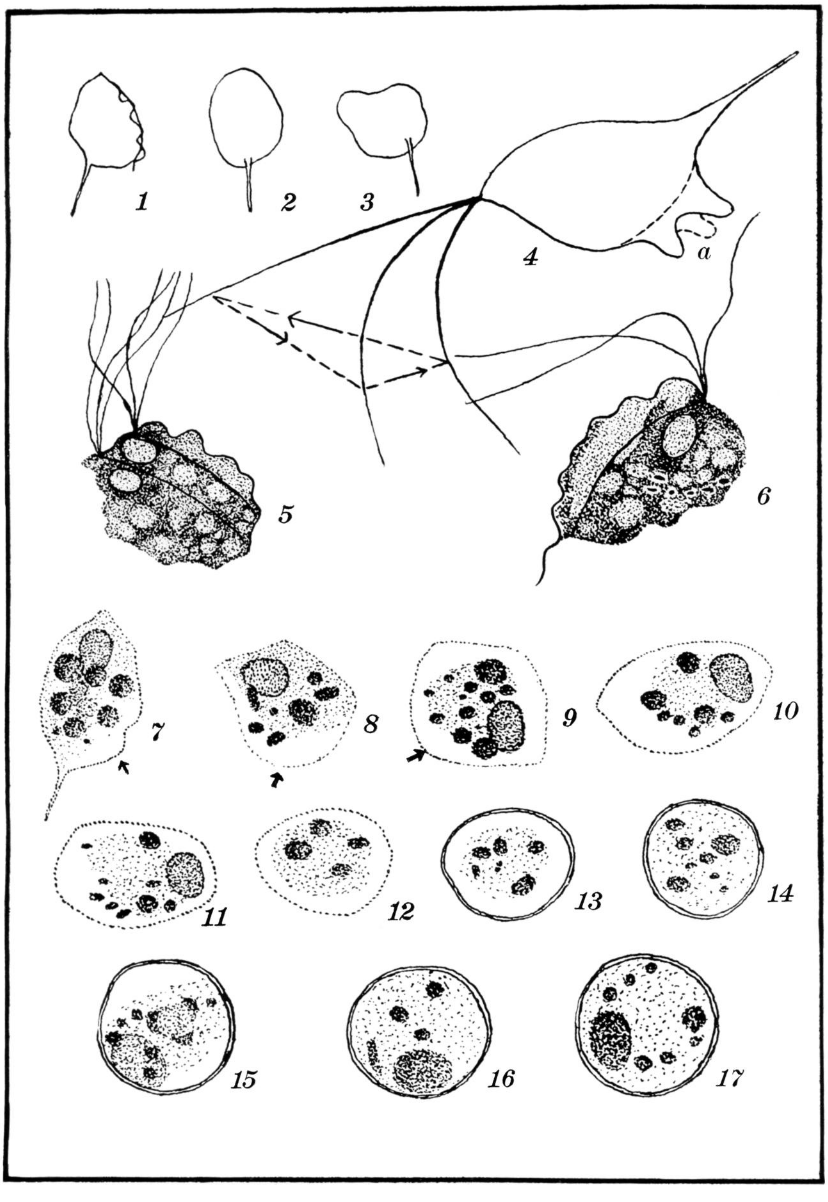

PLATE XI